

APPLICATION
FOR
UNITED STATES LETTERS PATENT

TITLE: **MANUFACTURE OF A MICROSENSOR DEVICE AND A
METHOD FOR EVALUATING THE FUNCTION OF A LIQUID
BY THE USE THEREOF**

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SPECIFICATION

Manufacture of a microsensor device and a method for evaluating the function of a liquid by the use thereof

5 The field of the Invention

This invention relates to a device for detecting a trace amount of substance, particularly to a material recognizing device for detecting a bio-molecular or other organic material or the like with a high sensitivity and on a real time basis.

10

Related Art

A biosensor system as a means to monitor a biological function instantaneously has been intensively studied and developed for practical applications heretofore. The basic composition of a biosensor consists of a section for detecting a biological substance and a section for transducing a signal. A biological substance is complexed with the recognizing component of the biosensor, and ensures an ability to detect a bio-molecule, while the signal transducing section transduces a change obtained through the detection of a biological substance into an electric signal. There are many kinds of biological substances which can be detected on the basis of their molecular properties, and they include enzymes, antibodies, binding proteins, lectin, receptors, etc. *Examples of* ~~what comes first includes~~ *Include those* biological substances that have a molecule recognizing ability and/or catalyzing function. They include enzymes, complex enzyme systems, intracellular organelles, microorganisms, animal cells, plant cells, etc. The catalytic activity of these substances depend on the structure characteristic with enzymes, and can be approximated, in its

Other Examples

essence, by the kinetic equation by Michaelis and Menten. What comes

~~next includes~~ substances that have a molecule recognizing function,
and which form
~~and forms~~ a stable complex through a biological affinity. They include

antibodies, lectin, binding proteins, receptors, etc. The basic

designing of a bio-sensor proceeds with ~~an~~ attention paid to the above

properties. With the recent development of biotechnology, the range

of biological substances available for the biosensor has been widened,

and thus thermo-resistive enzymes, monoclonal antibodies or the like

have
~~come~~ to be available. To convert the data obtained through molecule

recognition into electric signals, physical parameter converting

elements such as electrochemical reactions, and an FET, thermistor,

piezoelectric element, surface elastic wave element, photodiode, etc.

have been utilized.

However, the above-described conventional biosensor devices have

technical problems as described below. Firstly, the method for

producing a thin film for molecular recognition includes methods based

on photoresistance, electrochemical polymerization, manufacture of an

LB film, etc. The method based on photoresistance consists of forming

a photoresistant film on the entire surface of an ISFET (ion sensitive

field effect transistor), exposing only gate parts by lithography, and

forming a highly affinitive molecule recognizing film (organic film

or biomolecular film) on a gate insulating film. Then, the

photoresistant layer is peeled off to leave the molecule recognizing

film bonded to gate parts, which serves as a sensor. With this method,

however, it is difficult to neatly prepare minute dot electrodes on

the molecule recognizing film, *thus the incompletely*
~~and thus the incompletely~~ finished edge

of dots results. A reduced yield occurs. Further, waste of materials

occurs as a result of lithography. Namely, 99% of photo-setting resin

is discarded without being incorporated into actual products, that is, the method causes a wasteful consumption of resources on earth, and contamination of natural environments. This is a big problem. LB technique (Langmuir-Blodgett's technique) is a method whereby a

5 mono-molecular film is formed on the surface of water, and the film is transferred onto the surface of a solid substrate, and for the method to be effective, it is necessary for the mono-molecular layer to have a structure comprising hydrophobic and hydrophilic sections in a balanced state. This method, however, is problematic in that the

10 quality of LB film produced thereby is unsatisfactory in reliability: the film has immeasurable flaws or pores thereupon, and does not allow the formation of an uniform molecular film. Accordingly, with the product manufactured by this method, it is difficult to distinguish a change detected by a molecule recognizing film formed on an electrode

15 from a local change of the electrode.

Furthermore, the sensor film prepared by these methods is a molecule recognizing film composed of one kind of molecule, which recognizes only one kind of biological substance to which the film is sensitive. Still further, it is impossible with these methods to apply

20 different biological substances simultaneously to a plurality of electrodes. Thus, they are problematic in operability and detection efficiency.

With a view to cope with above-described inconveniences, this invention aims at introducing a method for producing a molecule

25 recognizing film distinct from the conventional ones, and further to introduce a method being different, in the manner of detecting biological substances, from the conventional ones.

One
 Namely, ~~the first~~ object of this invention is to provide a method for forming, distinct from conventional methods, a molecule recognizing film, uniform and high in quality on a sensor electrode efficiently and in a short time. Further, *another* ~~the second~~ object of this invention is to provide a method for forming a plurality of minute sensor electrode dots by said new method for preparing a molecule recognizing film, and for accurately applying a great number of biological samples to be evaluated onto said plural minute sensor electrode dots in a short time and efficiently.

10 *Summary*
 Brief description of the ~~Invention~~

one aspect of
~~According to this invention, with a sensor device comprising organic thin films formed on an arbitrarily chosen electrode board circuit and electrodes, and a transducing element to transduce information obtained by the organic thin films into electric signals, provided is a method for producing the sensor device wherein a solution of a material of the thin film is accurately printed via an ink-jet nozzle as micro-dots onto the required surface of microelectrodes so that the organic thin films are formed on the electrodes, thereby~~
 15 realizing highly dense microelectrodes.

another aspect of
~~According to this invention, provided is the sensor device as described in claim 1 wherein the solution of a material of the thin film comprises an electro-conductive polymer dissolved in a solvent.~~

another aspect
~~According to this invention, provided is the sensor device as~~
 25 described in claim 1 wherein the solution of a material of the thin film as described in claim 1 comprises a solution of a silicone-based surface modifying agent, or a mixture thereof with a solvent.

another aspect

According to this invention, provided is the sensor device as described in claim 1 wherein the solution of a material of the thin film as described in claim 1 comprises a mixture resulting from dissolution of a thiol compound in a solvent, and gold thin films are

5 formed on the surface of the electrodes.

another aspect

According to this invention, with said sensor device, provided is a method for evaluating a trace amount of liquid wherein a solution of a sample substance to be sensed is ejected into air as micro-dots via an ink-jet nozzle to fall on the surface of organic thin films of

10 microelectrodes so that the substance is submitted to evaluation.

another aspect

According to this invention, with said sensor device, provided is a method for evaluating a trace amount of liquid wherein the solution or liquid substance to be sensed and ejected into air as micro-dots via the ink-jet nozzle as described in claim 5 comprises a protein,

15 DNA, antibody, receptor, lectin, a bio-molecule from an animal or plant cell, or a physiologically active substance, or an aqueous solution thereof.

another aspect

According to this invention, provided are the sensor device and the method for evaluating the function of a liquid wherein the

20 electrode or electric circuit is formed on a plastic substrate,.

another aspect

According to this invention, provided are the sensor device and the method for evaluating the function of a liquid based on the use of the sensor device wherein the electric circuit is composed of poly-silicon thin film transistors,.

25

Brief Description of the Drawings

Fig 1 illustrates

~~Fig. 1 gives a diagram to illustrate~~ how minute electro-conductive polymer electrodes are formed by the method of this invention based on the use of an ink-jet.

Fig 2 illustrates

~~Fig. 2 gives a diagram to illustrate~~ how a functional solution sample is analyzed by the method of this invention based on the use of an ink-jet.

Fig 3 illustrates

~~Fig. 3 gives a diagram to illustrate~~ how a plurality of functional solution samples are analyzed in a short period on electro-conductive polymer electrodes by the method of this invention based on the use of an ink-jet.

Fig 4 illustrates

~~Fig. 4 is a block diagram to illustrate~~ the principle underlying the assay method of the microsensor device of this invention.

Fig 5 Shows

~~Fig. 5 gives an electronic circuit to collect data for analysis~~ using a microsensor device of this invention.

Fig 6 Shows

~~Fig. 6 gives an electronic circuit to collect data for analysis~~ using another microsensor device of this invention.

Fig 7 illustrates

~~Fig. 7 is a diagram to illustrate~~ how sensor thin membranes having various detection activities are formed on the microelectrodes of a microsensor device of this invention.

Description of the Embodiment

One embodiment

~~The smallest embodiment necessary for representing~~ the present invention will be described below with reference to attached figures.

Figs. 1, 2, 3, 4, 5 and 6 ^{show} give parts of interest to illustrate the structure of a sensor device embodying the present invention. Fig. 1 gives a schematic view of an ink-jet head: 10 stands for an ink-jet head; 11 for a head nozzle for ejecting ink droplets; 12 for an electro-conductive polymer applied on the surface of an electrode; 13

for TFT microelectrodes; and 14 for suspended ink droplets ejected via the ink-jet nozzle. The ink-jet head is driven by a piezo-electric element activating mode, whereby, when an electric signal is delivered from a driving circuit to the piezo-electric element, the piezo-electric element is deformed; a liquid within is pushed out by the deforming pressure; and the liquid is ejected via the nozzle.

In this case, the solution of an electro-conductive polymer is ejected via the ink-jet nozzle as micro-dots, and the dots of polymer solution in suspension are allowed to accurately reach the patterned microelectrodes to rest there. The usable electro-conductive polymer, for example, includes polypyrrol, polymethylpyrrol, polythiophene, polymethylthiophene, polyaniline, polyphenylene vinylene, or the like. Preparation of the solution of an electro-conductive polymer requires more or less modifications according to the property of the polymer, because some polymers dissolve in organic solvents while others do not. For an example take polypyrrol as the polymer of illustration. A THF (tetrahydrofuran) solution of 0.30mmol pyrrol, and another THF solution containing 0.25mmol phosphor monobutamate as a catalyst and 30mg of plastisizer are introduced into respective ink-jet tanks by nitrogen purge, and these solutions are ejected onto microelectrodes. Dots composed of two different kinds of solutions are mixed on an electrode to form a mixture there; the mixture is allowed to rest at room temperature for one hour to vaporize the solvent; and a solidified polymer thin film is formed there. Then, the surface of thin membrane is washed with THF or methanol; and residual solvent and unreacted monomers are removed. An electro-conductive polymer soluble in the organic solvent is dissolved in an organic solvent to a concentration at which the solution has a

viscosity of 3cps or less, is then introduced in an ink-jet tank to be ejected. To confer a selective absorbing activity to the electro-conductive polymer, material including a specific type of enzyme molecule or antibody chemically bound to the polymer, artificially synthesized molecule having similar recognizing function to them, or material that a special type of enzyme molecule or antibody is mixed into the electro-conductive polymer, are either dissolved in the solution to give a homogenous solution. Micro dots of various kinds of electro-conductive polymers resulting from polymer/enzyme or polymer/antibody mixtures are ejected via ink-jet nozzles to be printed; the solvent is allowed to vaporize; and a biosensor film results which carries an immeasurable number of sensor dots. Changes in the electric impedance of, or in the electric current through individual microelectrodes covered with respective electro-conductive polymers differ from each other according to the effects exerted by films resulting from agglutination of bio-molecules such as binding proteins, antibodies, DNAs, receptors, etc. adsorbed to the surface, and thus to find what substance is on a given electrode dot is easy. Fig. 2 gives a schematic view of the ink-jet to eject the sample solution onto the surface of individual microelectrodes: 21 stands for the ink-jet head; 22 for nozzles; 23: for microelectrodes; 24 for sample solution; and 25 for suspended droplets of sample solution. In the same manner by which the first molecule recognizing films were formed on the electrodes, micro-dots comprising the sample solution were ejected via the ink-jet nozzle into air and printed on the molecule recognizing films made of an electro-conductive polymer. By virtue of the biomolecular film thus formed on the electro-conductive polymer, changes in electric impedance or in minute

electric current through individual electrodes are detected, which allows a quick evaluation/analysis of a huge number of samples

For example, production of a patterned array of microelectrodes is possible by ejecting a plurality of electro-conductive polymer solutions via a multi-line head nozzle into air, and thereby forming, for example, ten different kinds of sensor dots each comprising several hundreds line dots.

Fig. 3 is a diagram to illustrate how plural lines of dots comprising different electro-conductive polymers are formed on the surface of a substrate like the one as used for the semiconductor circuit board, to form a two-dimensional sensor, and how biological sample dots are formed thereupon to be stabilized there. Assume, as shown in the figure, in a crosswise direction, five different electro-conductive polymers are placed one after another five times in repetition to produce 25 dots in total. These unit arrays are placed in the same manner repetitively in the lengthwise direction to produce a panel of 25 unit arrays which occupies practically the entire surface of substrate. Upon this unit array of microelectrode sensor dots, this time, five different kinds of biological samples are ejected via the ink-jet nozzle in the crosswise direction one after another five times in repetition until 25 micro-dots are suspended in air to fall onto respective electrodes. Then, by monitoring changes in the adsorption of biological material to the electrode, it is possible to assay/evaluate the biochemical characteristics and responsiveness of respective biological samples quickly on a real time basis, and repeatedly and simultaneously. In this particular example, for each of 25 different combinations, data comprising 25 measurements can be obtained. As another example, let's assume a case where an array of

micro-dot electrodes comprising ten different kinds of electro-conductive polymers is prepared, and ten different kinds of biological samples are prepared so that they correspond with these dot electrodes. Then, simultaneous measurements based on 100 different combinations become possible. Assume that this forms a unit array, and that the unit arrays are repeated five times to form the same panel as above. Then, it is possible to obtain 25 times repeated measurements for each of 100 different combinations

Then, the assay dependent on the use of a sensor device array produced in the manner as described above will be described. Fig. 4 gives a simple block diagram of a circuit responsible for the assay dependent on the use of a resistor sensor array. Principal functions depicted in the figure are roughly represented by a resistor sensor multiplex section, signal processing circuit section and pattern recognizing section. Namely, the function depicted in this figure ~~consists of~~ ^{includes} picking up signal from a single channel out of the multi-channel resistors, and processing and recognizing it. Accordingly, it is firstly important to accurately detect a change in impedance.

The simplest and most accurate way of determining a resistance includes various bridge methods, but these methods are not suitable for measuring a change in resistance. An alternative method includes a resistance to frequency conversion. This method, however, is disadvantageous in that it is accompanied by ~~noises~~ ^{noise}, and requires a rather long time for measurement. As one general method for detecting a change in resistance, a circuit working on a voltage mode as shown in Fig. 5 has been known. In this circuit, a specific type of resistance sensor is chosen; a constant current is ~~flowed through~~ ^{applied to} it; and the

voltage across the sensor is monitored. Then, as the voltage varies in proportion to the resistance, it is possible to detect a change in resistance by following a change in voltage. To determine changes in resistance it is advisable to subtract the voltage given as a base to the sensor by means of a differential amplifier, and then to amplify the differential signal with a high-gain amplifier. The sensitivity of the circuit depicted in Fig. 5 is proportional to the gain of amplifier, and is given by the following equation:

$$V_o = A (I_s R_s - V_{off}),$$

where $(\delta V_o / \delta R_s) = A I_s$.

An alternative method by which to detect a change in resistance includes a method working on a current mode. Fig. 6 gives a circuit diagram of the method. In this figure, a constant voltage is applied to a resistor sensor chosen for this purpose. To measure a change in resistance, a constant current supplied from a source is ~~flowed through~~ ^{applied to} the sensor as an offset current; differences in current are removed as a signal; and the signal is amplified. The sensitivity of the circuit is proportional to the current gain of amplifier and to the resistance of sensor.

$$I_o = A(I_{off} - V_s / R_s)$$

where

$$(\delta I_o / \delta R_s) = A_s / R_s^2 = A I_s / R_s$$

This type of current detection method commands a higher degree of freedom than does the voltage detection method, and thus simplifies the subsequent processing of signals.

~~Above-described~~ ^{The above} semiconductor circuits are usually constituted of field effect transistors (FET) arranged on a monocrystal silicon substrate. However, because in recent years the function of thin film

transistors (TFT) formed on a polycrystal silicon (P-Si) film has made a notable progress, it becomes possible to prepare this type of circuit using polycrystal Si thin film transistors (P-Si TFT). The P-Si TFT has advanced so much that its function is essentially equal to that of monocrystal FET. Further, introduction of the method enabling the manufacture of polysilicon at a low temperature allows the use of a spacious glass substrate. This brings about a great cost-reduction and a method that is suitable for the production of sensor devices like *described herein* the one of this invention.

TFT microelectrodes can be formed not only on a glass substrate but on a thin plastic substrate having a softness and flexibility.

The TFT microelectrodes formed on such substrate can recognize or act upon not only liquid samples, but various gas molecules and volatile substances. Namely, individual volatile molecules are adsorbed to the surface of electro-conductive polymers; the electric resistance of electro-conductive films changes in association; and the change is transformed into an electric signal for detection. For example, the sensor device can be used for the recognition of ethanol, acetone, harmful gases such as chlorine gas, cyanide gas, etc., or vapours of aromatic molecules including perfumes and other odourants.

To be precise, this sensor device in film is left in an atmosphere comprising a gas or vapour; the adsorbed gas molecule penetrates through the surface of electro-conductive polymer into its interior; and the entire polymer film changes its volume and/or other property after undergoing expansions/contractions to cause changes in electric resistance of the film. These changes are transformed into electric signals for detection, and fed into a computer, and the information

obtained through recognition thereof is available for a quick feedback.

Several sensor arrays ^{formed} ~~consisting~~ ^{the simultaneous} of field effect TFTs could be used for ~~the simultaneous~~ identification, classification and

5 quantification of odours or other molecules. These sensors could be used in conjunction with other sensors to detect chemicals. The TFT sensors could be integrated onto a single device. Different electro-conductive polymers are placed upon the gates of the TFT sensors using ink-jet technology. In another modification the array
10 of TFTs could be constructed in a pattern to produce a two-dimensional map of the odour response. The output from each TFT would then resemble the output from a pixel of a CCD camera. Different conducting polymers could be deposited onto different regions of the array to produce a device which was specific to a group or class of chemicals, e.g.
15 aromatics. When the molecules bind to or react with the polymer a two-dimensional map corresponding to the particular odours will appear.

The TFT chip should include driving and reading electronics. The information of the odour molecule could be read directly or
20 processed using for example neural networks or classical image processing techniques to identify the odour molecules high sensitively. The density and information of a special type of molecular composition of different odours could thus be obtained.

Fig. 7 is a diagram illustrating how sensor thin films possessed
25 of various detection functions are prepared on the microelectrodes formed on a sensor thin film: 71 stands for an electro-conductive polymer film; and 72 for electrodes A and B. The size of each microelectrode is preferably in the range of 1-100 μ m. The sensor is

stabilized on the polymer solution electrode which has been ejected via an ink-jet nozzle, and converted to a thin film. A bio-molecule or the like is adsorbed to the surface of this electro-conductive polymer film; and a change in resistance or in current generated as a result of the adsorption is monitored by the above-described detection method.

Alternatively, a silicon-based, functional, surface modifying solution is ejected via an ink-jet nozzle to be applied onto the surface of a microelectrode, to form a silicone-based, functional molecular film there; a bio-molecule is chemically adsorbed to that film to cause thereby electrons within to move towards the surface of electrode; and therewith it is possible to selectively detect the substance adsorbed to the electrode surface. By the use of a device with basically the same in composition with that as depicted in Fig. 7, that is, a device wherein a silicon-based, functional molecular film is formed on an electrode, and an electron-mobile protein molecule such as cytochrome C is bound or adsorbed to that film, it is possible to monitor the adsorption of protein to the surface of electrode by following minute current changes resulting from electron transfer from the protein.

Or, it is possible to plate a gold thin film onto the surface of a microelectrode such that a thiol molecule and gold interact with each other to form a self-organizing agglutination, which results in the formation of a functional, monolayer film. The functional group projecting from the surface of thiol monolayer which has been generated as a result of self-assembly on the gold thin film plated on the microelectrode has a function to selectively recognize a specific bio-molecule or a volatile molecule. For example, as the functional group projecting from the thiol molecule, a biotin derivative may be

used. A biotin molecule has a strong binding activity towards a specific binding site of avidin or streptavidin, and its binding constant is about 10^{15} . This is practically the same as that encountered in a covalent bond. To this biotin molecule film is transferred, for example, a solution of avidin-ferritin binding protein via an ink-jet nozzle. Then, avidin and biotin are selectively adsorbed; and the ferritin protein molecule is stabilized on the electrode. The thus selectively adsorbed molecule causes a change in refractive index of the entire molecular film, and that change is captured as a change in dielectric constant of the adsorbing molecular film. Namely, it is possible to convert the microelectrode into a polarized thin Film (capacitor) ~~film (capacitor)~~, which serves as a sensor.

Advantages

According to this invention, provided is a method by which, in contrast with conventional ones, a molecule recognizing film is efficiently and in a short period formed on a microsensor in a uniform and high quality manner. Further, according to this invention, provided is a method by which to accurately introduce a vast number of biological samples to be evaluated in a short period and efficiently to plural, minute sensor electrode dots which have been prepared according to said method for the formation of a molecule recognizing film.